AMENDMENTS TO THE SPECIFICATION

IN THE SPECIFICATION:

Please amend the paragraph on page 2, line 13 as follows:

According to this publication, the IPCS sequence is defined as a degenerated motif replying to the sequence AAATGRYKKCMMS (SEQ ID NO: 1) (IUAP code) which sequence has been shown according to said publication to bind a nuclear factor denoted IPCS-binding factor (IPCS-BF). Said IPCS-BF factor is composed of a single polypeptide of 26 kDa which is present constitutively in nuclear extracts of U937 cells and Peripheral Blood Mononuclear cells from healthy donors. According to the publication of Lallemand C. et al, it is believed that the transcriptional regulation of the p53 and IRF1 genes is influenced by the binding of said IPCS-BF factor, to the IPCS sequence.

Please amend the paragraph on page 3, line 24 as follows:

The invention thus relates to the use of a nucleotide sequence selected among:

(i) a nucleotide sequence comprising the DNA sequence identified ~~under No. 1~~as SEQ ID NO: 38, and represented on Figure 14A, or ~~under No. 3~~SEQ ID NO: 44 and represented on Figure 14B ;

(ii) a nucleotide sequence encoding a polypeptide having the amino-acid sequence identified ~~under No. 2~~as SEQ ID NO: 40, and represented on Figure 15A or encoding a polypeptide having the amino-acid sequence identified ~~under No. 4~~as SEQ ID NO: 46 and represented on Figure 15B;

(iii) a nucleotide sequence comprising the DNA sequence identified ~~under No. 5~~as SEQ ID NO: 45, and represented on Figure 14C ;

(iv) a nucleotide sequence encoding a polypeptide having the amino-acid sequence identified ~~under No. 6~~as SEQ ID NO: 47, and represented on Figure 15C;

(v) a nucleotide sequence derived from sequence defined under (i), (ii), (iii) or (iv) wherein said sequence is modified particularly by deletion, addition or substitution of one or more nucleotides providing that the resulting nucleotide sequence encodes a polypeptide capable of binding a nucleotide sequence designated IPCS which comprises the DNA sequence AAATGNNNNC (SEQ ID NO: 2), wherein N means any nucleotide (G, A, C or T(U))

for the expression in a determined eucaryotic cell, of a polypeptide capable of interacting with said nucleotide sequence designated IPCS and capable of acting as a positive transcriptional factor for the transcription of a nucleotide sequence placed under the control of said IPCS sequence and present in said eucaryotic cell.

Please amend the paragraph on page 5, line 1 as follows:

Particular IPCS sequences have been found in the p53 and IRF1 genes, which have the following DNA sequence: AAAATGATTTCCAC (SEQ ID NO: 3) (for the p53 gene) and GAAATGACGGCACG (SEQ ID NO: 4) (for the IRF1 gene). This sequence has also been identified in other anti-oncogenes such as Rb, p21 (WAF1), p27, wt1, bax, TNF receptor and FAS genes.

Amend the paragraph on page 5, line 6 as follows:

These IPCS sequences are described hereafter by reference to the gene containing the same.

Gene	Pos.	Sequence IPCS	SEQ ID NO	Ref. genebank
Rb	680	aaatgtattc	5	L11910
P21	1138	aaatgtattc	5	U24170
P21	1876	aaatgaaaac	6	U24170
P21	2031	aaatggtgac	7	U24170
P21	4314	aaatgtgtcc	8	U24170
P27	292	aaatggcaac	9	AB003688
Wt1	1828	aaatgggctc	10	X74840
Bax	679	aaatggtgcc	11	U17193
Bax	694	aaatgaaggc	12	U17193
TNFr	946	aaatgaacac	13	U53483
FAS	580	Aaatgtcaac	14	U31968

Please amend the paragraph on page 5, line 21 as follows:

The DNA sequences identified ~~under No.1, No. 3 and No. 5~~as SEQ ID NOS: 38, 44 and 45 are the sequences presented respectively in Figures 14A, 14B and 14C and designated respectively GAAP-1 and GAAP-2.

Please amend the paragraph on page 5, line 24 as follows:

The aminoacid sequences identified ~~under No 2., No. 4 and No. 6~~as SEQ ID NOS: 40, 46, and 47 are presented respectively in Figures 15A, 15B and 15C and designated respectively GAAP-1 and GAAP-2.

Please amend the paragraph on page 7, line 28 as follows:

According to another particular embodiment of the invention, the nucleotide sequence which is used codes for a GAAP-1 polypeptide comprising the amino acid sequence identified ~~under No. 2~~as SEQ ID NO: 40 or for a GAAP-1 polypeptide comprising the amino acid sequence identified ~~under No. 4~~as SEQ ID NO: 46.

Please amend the paragraph on page 8, line 9 as follows:

GAAP-2 can be considered as a variant of GAAP-1, by addition of 23 specific amino-acid residues translated from exons III and IV having additional nucleotide residues as a result of alternative splicing. The additional 23 amino-acid residues form a polypeptide having the following sequence with respect to the sequence of figure 15A:
MGQKFQKKKSYRLVLKELRNPLK (SEQ ID NO: 42).

Please amend the paragraph on page 9, line 25 as follows:

The invention also relates to a nucleotide sequence comprising the DNA sequence identified ~~under No. 1, or under No. 3~~as SEQ ID NO: 38, or

SEQ ID NO: 44, said nucleotide sequence being devoid of the sequence forming exon IV in the PRDII-BF1 gene.

Please amend the paragraph on page 10, line 1 as follows:

In a particular embodiment of the invention, this nucleotide sequence consists of the DNA sequence identified ~~under No. 1 or under No. 3~~as SEQ ID NO: 38 or SEQ ID NO: 44 (Figure 14A or Figure 14B).

Please amend the paragraph on page 10, line 4 as follows:

The invention also relates to a nucleotide sequence comprising the DNA sequence identified ~~under No. 5~~as SEQ ID NO: 45, said sequence comprising 69 nucleotides in addition to that of ~~Sequence ID No. 1~~SEQ ID NO: 38.

Please amend the paragraph on page 10, line 7 as follows:

In a particular embodiment of the invention, this nucleotide sequence consists of the DNA sequence identified ~~under No. 5~~as SEQ ID NO: 45 (Figure 14C).

Please amend the paragraph on page 10, line 23 as follows:

Particular nucleotide sequences derived from the above defined nucleotide sequences are those selected from the group consisting of:

(i) a fragment of the DNA sequence identified ~~under No. 1~~as SEQ ID NO: 38 (Figure 14A), or a fragment of the DNA sequence identified ~~under~~

~~No. 3~~as SEQ ID NO: 44 (Figure 14B) which can be used as a specific probe to detect the presence of said DNA sequence identified ~~under No. 1, No. 3, or No. 5~~as SEQ ID NOS: 38, 44, or 45 or a mutated sequence thereof,

(ii) a nucleotide sequence encoding a polypeptide having the amino-acid sequence identified ~~under No. 2~~as SEQ ID NO: 40, or a nucleotide sequence encoding a polypeptide having the amino-acid sequence identified ~~under No. 4~~as SEQ ID NO: 46 or a nucleotide sequence encoding a polypeptide having the amino-acid sequence identified ~~under No. 6~~as SEQ ID NO: 47,

(iii) a nucleotide sequence derived from sequence defined under (i) or (ii) wherein said sequence is modified, especially by deletion, addition or substitution of one or more nucleotides providing that the resulting nucleotide sequence encodes a polypeptide capable of binding a nucleotide sequence designated IPCS which comprises the DNA sequence AAATGRYKKC (SEQ ID NO: 15), and is capable when used in appropriate conditions, of expressing in a determined eucaryotic cell, a polypeptide interacting with the nucleotide sequence designated IPCS and acting as a positive transcriptional factor for the transcription of a nucleotide sequence placed under the control of said IPCS sequence and present in said eucaryotic cell.

Please amend the paragraph on page 13, line 26 as follows:

A preferred recombinant polypeptide according to the invention comprises the amino acid sequence identified ~~under No. 2~~as SEQ ID NO: 40

or ~~under No. 4 or under No. 6~~SEQ ID NO: 46 or SEQ ID NO: 47 (Figures 15A, 15B and 15C).

Please amend the paragraph on page 14, line 1 as follows:

Another particular polypeptide according to the invention has an aminoacid sequence shorter, which is comprised within the ~~the~~ amino acid sequence identified ~~under No. 2~~as SEQ ID NO: 40, or ~~under No. 4, or under No. 6~~as SEQ ID NO: 46, or as SEQ ID NO: 47 or a variant thereof replying to the definitions given above, provided the obtained polypeptide is capable of interacting with the nucleotide sequence designated IPCS to act as a positive transcriptional factor for the transcription of a nucleotide sequence placed under the control of said IPCS sequence and present in a eucaryotic cell.

Please amend the paragraph on page 14, line 8 as follows:

The invention also relates to the polypeptide having the following sequence: KSYRLVLKELRNPLKR (SEQ ID NO: 43). This polypeptide can be used as antigen for the preparation of an antibody which would recognize GAAP-2 and would not cross react with PRDII-BF1 and GAAP-1.

Please amend the paragraph on page 15, line 5 as follows:

The recombinant eucaryotic cell according to the invention can also be defined as a cell which is recombined with a nucleotide sequence encoding a polypeptide capable of binding a nucleotide sequence designated

IPCS and comprising the DNA sequence AAATGNNNNC (SEQ ID NO: 2), for the expression in a determined eucaryotic cell of a polypeptide capable of interacting with the nucleotide sequence designated IPCS and capable of acting as a positive transcriptional factor for the transcription of a nucleotide sequence placed under the control of said IPCS sequence and present in said eucaryotic cell.

Please amend the paragraph on page 15, line 13 as follows:

In a particular recombinant eucaryotic cell according to the invention, the polypeptide comprises the amino acid sequence identified ~~under No. 2~~ as SEQ ID NO: 40 ~~or under No. 4 or under No. 6~~or as SEQ ID NO: 46 or as SEQ ID NO: 47 (Figures 15A, 15B and 15C).

Please amend the paragraph on page 18, line 19 as follows:

The invention therefore relates to a process for the in vitro detection of a deficient BRDII-BFI gene comprising the steps of:

- contacting a probe constituted from the nucleotide sequence identified ~~under No. 1 or under No. 3, or under No. 5~~as SEQ ID NO: 38 or SEQ ID NO: 44 or SEQ ID NO: 45, or a fragment thereof comprising the zinc finger binding domains corresponding to the domains localised within exon VI of the BRDII-gene, with the DNA of a cell normally constitutively expressing said gene, in stringent conditions,
- detecting the hybridisation product between said probe and said cell DNA.

Please amend the paragraph on page 18, line 27 as follows:

One appropriate probe can be the nucleotide sequence spanning exon 5 to exon 9, i.e., nucleotides 6396 to 9020 of the sequence identified ~~under No. 1~~as SEQ ID NO: 38.

Please amend the paragraph on page 20, line 26 as follows:

Figure 1A Schematic representation of the reporter construct used to clone IPCS-BF1. The IPCS from p53 (SEQ ID NO: 3) or IRF1 (SEQ ID NO: 4) gene or the mutated IPCS (SEQ ID NO: 48) is cloned upstream His3 minimum promoter.

Please amend the paragraph on page 21, line 10 as follows:

Figure 3 - Deduced amino acid sequence of IPCS-BF1 (SEQ ID NO: 34). The putative NLS, the two zinc fingers domain and the PEST domain are framed.

Please amend the paragraph on page 21, line 24 as follows:

Figure 5c) Amino acid sequence of the two zinc finger domains. GAAP-1, GAAP-1 mtZ1, and GAAP-1mt Z2 are shown as SEQ ID NOS: 35, 36, and 37, respectively. The residues which are substituted in GAAP-1 mtZ1 or in GAAP-1 mtZ2 are underlined.

Please amend the paragraph on page 22, line 25 as follows:

Figure~~s~~ 14A~~ and 15A~~- GAAP-1 and PRDII sequences (SEQ ID NOS: 38 and 39)

Figure 15A- GAAP-1 and PRDII sequences (SEQ ID NOS: 40 and 41)

Please amend the paragraph on page 22, line 26 as follows:

Figures ~~14B and 15B~~ variants of GAAP-1 nucleotide sequence (SEQ ID NO: 44) ~~and amino acid sequences.~~

Figure 15B- variants of GAAP-1 amino acid sequence (SEQ ID NO: 46)

Please amend the paragraph on page 22, line 28 as follows:

Figures ~~14C and 15C~~ - GAAP-2 nucleotide sequences (SEQ ID NO: 45)

Figure 15C- GAAP-2 amino acid sequence (SEQ ID NO: 47)

Please amend the paragraph on page 25, line 6 as follows:

Cloning of GAAP-1

S. cerevisiae YM4271 and the reporter vector pHISi were obtained from Clontech. The reporter constructs were generated by inserting one copy of the double stranded oligonucleotides 5'AAAATGATTTCCAC3' (SEQ ID NO: 16), 5'-AAAACGATTTCCAC-3' (SEQ ID NO: 17), 5'-GAAATGACGGCACG3' (SEQ ID NO: 18), corresponding respectively to the IPCS-p53, IPCS-p53M and IPCS-IRF1 sequences previously identified (Lallemand 97), into the EcoRI and XbaI sites of the pHISi. These plasmids were linearised and used to transform YM2471 competent cells. The yeast reporter strains were maintained by selection on synthetic dextrose medium lacking histidine. As each yeast reporter strain possess its own leaky expression of HIS3, 20mM, 30mM and 45mM of 3-AT (3-amino-1,2,4-triazole) were added to the medium to suppress the background growth of respectively IPCS-p53, IPCS-IRF1 and IPCS-p53m yeast reporter strains. Screening of the human leukocyte cDNA library

(Clontech) encoding proteins fused with the GAL4 activation was performed by the lithium acetate method (Gietz 92) in a yeast strain carrying the HIS3 reporter gene under the control of one IPCS-p53 sequence. The transformed yeast cells were plated under selective conditions in synthetic medium containing 20mM 3AT and lacking histidine and leucine. In order to confirm the transactivation properties of positive clones, plasmids were recovered from these clones by a rapid isolation procedure (Kaiser & Auer 1993), and used to transform E. coli. A mini-preparation (Wizard, Promega) of these plasmids were then used to transform yeast strains carrying the HIS3 reporter gene under the control of the IPCS-p53, IPCS-IRF1 or IPCS-p53M sequences. Only plasmids which can confer auxotrophy to histidine to strains containing IPCS-p53/HIS3 and IPCS-IRF1/HIS3 and non IPCS-p53M were retained.

Please amend the paragraph on page 26, line 12 as follows:

Plasmids

The GAAP-1 expression vector was constructed by cloning the PCR product (nt 6396 to 8601) from human leukocyte cDNA library (Clontech Laboratories) in the pcDNA3.1 expression vector (Invitrogen) using the following primers: 5'-AGCATGGCATTAGGTAATCAAAAGTCCACAG-3' (SEQ ID NO: 19) 5'CCATCAGGTTGCTATCACAAGC-3' (SEQ ID NO: 20). The plasmid expressing the EGFP/GAAP-1 fusion protein was obtained by fusion PCR (Higuchi, R., In PCR protocols, a guide to methods and applications (Eds Innis, M.A.,

Gelfand,,) using the following primer couples (5'-ACCATGGTGAGCAAGGGCGA-3' (SEQ ID NO: 21), 5'-GATTACCTAATGCTCTCTTGTACAGCTCG-3') (SEQ ID NO: 22) and (5'-GGACGAGCTGTACAAGATGGGGCAGAAGTTTCAAAA-3' (SEQ ID NO: 23), 5' CCATCAGGTTGCTATCACAAGC -3' (SEQ ID NO: 24)) to amplify respectively the EGFP and GAAP-1 coding region. The EGFP/GAAP-1 del NLS, the GAAP-1 mtZ1 and the GAAP-1 mtZ2 were obtained by overlapping PCR (Higuchi above) using the following overlapping primer couples (5'-CCTTAATCAAAAGTGAAGATGGAGGATATAAGTCA-3' (SEQ ID NO: 25), 5'-ATCCTCCATCTTCACTTTTGATTAAGGAATT-3' (SEQ ID NO: 26)7), (5'-GCAGAAGAAGCTGGAATACGTTGTAAGAAAC-3' (SEQ ID NO: 27), 5'-AGCTTCTTCTGCAATGTATTTTCCTCTTCC-3' (SEQ ID NO: 28)) and (5'-GCAATGAAGTCCAAGGCAGGAAGCAAGAAATGTGTGGA-3' (SEQ ID NO: 29), 5' TCCTGCCTTGGACTTCATTGCTTTTGTCAGATTTCC-3' (SEQ ID NO: 30)) ~~respecitvely.~~ respectively. All the coding regions were cloned in the expression vector pcDNA3.1. (Invitrogen). The integrity of the constructs was verified by sequencing. The pRL-SV40 was purchased from Promega (Promega).

Please amend the paragraph on page 28, line 15 as follows:

EMSA

Synthetic double stranded oligonucleotide probes were labelled with a-32P dCTP (Amersham Corp.) by "filling-in" with Sequenase, and then purified on Sephadex G-50. The protein-DNA binding reactions were carried out using 5 µg of nuclear extract and 0.1 fmol of probe in 20 µl of binding buffer (20 mM Tris-HCl, 25 mM KCl, 1 mM DTT, 1 mM EDTA, 0.1% Nonidet P-40,4%

glycerol, 1 mg/ml bovine serum albumin, µg/ml poly(dI-dC)) for 20 min at 4 °C. In vitro translation products were generated by 50µl of TNT coupled reticulocyte Lysate System (Promega) and 1µg of the appropriate plasmid. 5µl of the in vitro product was used instead of the nuclear extract when required. The reaction mixture was then electrophoresed on a 6% non denaturing acrylamide gel. Competition experiments were carried out using a 50-fold molar excess of the unlabelled probe. The following oligonucleotides (and their complementary strands) were used in these studies: IPCS-IRF1, 5'-AGCCTGATTTCCCCGAAATGACGGCACGCAGCC-3' (SEQ ID NO: 31);IPCS-p53, 5'-AATGCAGGATTCCTCCAAAATGATTTCCAC-3' (SEQ ID NO: 32); NF-kB-MHC 5'-GATCCTCTGGGGATTCCCCATGGA-3' (SEQ ID NO: 33).

Please amend the paragraph on page 29, line 23 as follows:

To identify complementary DNAs encoding proteins able to interact with the IPCS sequence we used a yeast one-hybrid system. A yeast tester strain, YIPCS, was established by introducing the reporter plasmid, pHISi-IPCS, into strain YM4271 (Clontech) (HisUraLeu). The pHISi-IPCS contained 14-bp of IPCS from the p53 promoter (IPCS-P53) corresponding to the sequence AAAATGATTCCAC (SEQ ID NO: 16), upstream of the minimal promoter of the HIS3 gene of the pHISI vector (Clontech laboratories). Strain YIPCS was transformed by a human leukocyte cDNA library (Clontech), in which cDNAs were fused with the GAL4 activation domain, and plated on medium lacking leucine and histidine and containing 20 mM 3-AT. From 107 transformants in this manner, we isolated a 2.5 kb cDNA clone coding for a

protein fused with the GAL4 activation domain. To control the specificity of the interaction of this protein with IPCS, we transformed yeast cell lines harbouring a HIS3 reporter gene under the control of different IPCS sequences (fig. 1A) with the 2.5 kb cDNA clone. The hybrid protein was able to up-regulate the HIS3 gene controlled by the wild type IPCS from p53 and IRF1 genes, but not the one controlled by a mutated IPCS (Fig 1B). This result suggests that the protein coded by the 2.5 kb cDNA clone possess binding properties for the IPCS sequence from the p53 promoter (nucleotides 6396 to 6488).

IN THE SEQUENCE LISTING:

Please replace the Sequence Listing of record with the Substitute Sequence Listing enclosed herewith.